MICROSTIMULATION OF LUMBOSACRAL SPINAL CORD-MAPPING

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Second Progress Report
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Neural Prosthesis Program

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This QPR is being sent to you before it has been reviewed by the staff of the Neural Prosthesis Program.

I. Introduction

During this quarter progress was made in three areas of investigation: (1) Recording of cavernous sinus pressure changes from the cat penis as a model for penile erection. From our preliminary studies using ventral root or pelvic nerve stimulation we determined that recording of cavernous sinus or corpus cavernous pressure changes correlated well with penile protrusion and tumescence. These pressure changes seem to be a good method of mapping and quantifying the response to spinal cord stimulation. An abstract to Society for Neuroscience is enclosed on this subject. (See attached abstract). (2) Tracing studies continued during this quarter using pseudorabies virus (PRV) to determine the location of penile efferent neurons and interneurons in the lumbosacral spinal cord as well as at supraspinal sites. An abstract to the Society for Neuroscience on tracing of penile neurons in the central nervous system is enclosed (See attached abstract). Tracing studies also continued during this quarter on bladder efferent neurons and interneurons in animals with selective nerve cuts. (3) During this quarter most of the hardware to hold the spinal cord and hindlimb rigid and to record torque for our motor studies was tested. An animal was placed in the spinal frame and a few additional modifications were made. The computer hardware and software for data collection, analysis and hard copy output is working and experiments should begin next quarter.

II. Changes in Penile Cavernous Sinus Pressure by Microstimulation of the Sacral Spinal Cord

During this quarter we continued our studies on sites in the sacral spinal cord which produce penile erection. Our cat animal model uses changes in penile cavernous sinus pressure as a quantative measure of penile tumescence or erection. The methods used in these studies were presented in detail in a previous progress report but are summarized here together with

some modifications made during this quarter.

Adult male cats anesthetized with pentobarbital (20-30 mg/kg iv) are used in these studies. We continue to use pentobarbital anesthesia in these experiments since ∝-chloralose used in our earlier bladder experiments seems to inhibit penile erection. Cavernous sinus pressure is recorded by a flexible 22 gauge iv catheter placed in the corpus cavernous via a small incision in the tip of the penis and secured with a suture. This method is now routinely used and seems to give stable pressure recordings for extended periods of time. Bladder pressure is also recorded in these animals via a polyethylene catheter placed in the dome of the bladder. Although under pentobarbital anesthesia spontaneous rhythmic bladder activity is usually not seen, stimulus induced bladder contractions are seen with ventral root or spinal cord stimulation. The amplitude of bladder contraction may be somewhat reduced with spinal cord stimulation however. A large laminectomy from L_4 to S_3 exposes the lumbosacral spinal cord and the dorsal and ventral roots. The dura is open to allow access to the spinal cord with fine tipped (200-400μ^2 exposed surface) activated iridium electrodes. In past experiments the exposed cord and roots were covered with warm mineral oil throughout the experiment. It was noticed that the responses to ventral root stimulation deteriorated over the duration of the experiment (usually 24-36 hours). In recent experiments we used a warm oxygenated Krebs' ringer solution to cover the exposed roots and spinal cord, which seemed to preserve, for a longer period of time, the responses seen from cord and root stimulation. One draw back of using a conductive solution such as Krebs' is that the threshold for ventral root stimulation is increased slightly. Microstimulation below the surface of the spinal cord is however not affected. We will continue to examine various types of bathing solutions for our experiments.

At the beginning of each experiment we stimulate each sacral ventral root to determine

the spinal segment which produces the largest amplitude penile pressure response and bladder response. In most instances the penile or cavernous sinus pressure response is greatest with S_1 ventral root stimulation while the bladder pressure response is largest with S_2 stimulation.

Following identification of the spinal level which gives the largest response to ventral root stimulation that segment of the spinal cord is mapped with activated iridium microelectrodes. Figure 1 shows electrode tracts which produced increases in cavernous sinus pressure to S_1 spinal cord focal stimulation at 25 Hz, 0.2 msec negative first pulses, 100-150μA, for one minute. Responses are seen over a small area in the lower to middle part of the ventral horn, 1.8 mm from the cord surface and half-way between the midline and lateral edge of the ventral grey. Sites dorsal or ventral to the active site often showed no or small responses. The stimulus parameters especially the frequency, duration and intensity of stimulation were important in producing a large response with a latency less than 30-40 seconds. In general the frequencies which gave the best responses were in the 25 - 35 Hz range and intensity of stimulation of 100-150μA (See Figure 2 & 3). The duration of stimulation was usually one minute. All three of these parameters were higher than those used to produce large bladder contractions to sacral cord stimulation (15-20Hz, 25-100μA, for 10 seconds). In general the latency to peak pressure was long (8-40 sec) compared to the bladder response (less than 2 sec). The duration of the penile response often, however, outlasted the stimulus. In some instances turning the stimulus off produced a rapid but small drop in penile pressure followed by a partial but sustained recovery. The pressure slowly returns to baseline over 30-60 seconds. The rapid, but small drop in pressure when the stimulus is turned off is probably a striated muscle response while the sustained response is a slower smooth muscle response. Further evidence that the rapid response is striated muscle, while slow response is due to a smooth muscle component, is that the

rapid relaxation is blocked by Pavulon, a neuromuscular blocking agent, while the smooth muscle contraction is not affected by Pavulon.

At the end of these experiments the catheter placed in the cavernous sinus is injected with dye to determine the position of the catheter tip. In addition the spinal cord is fixed with formalin, sectioned, stained and examined microscopically to identify electrode tracts. From these histological sections the position of the stimulation sites and responses are correlated (Figure 1). These types of studies will continue into the next quarter, with some additional mapping studies and with nerve and spinal cord transections to determine the contribution of penile reflexes to the observed responses.

III. Penile Efferent Neurons and Interneurons Revealed by Pseudorabies Virus Tracing Techniques

During this quarter we continued our studies using PRV to trace pathways in the central nervous system to bladder and penis. We were particularly interested in CNS pathways to the cavernous sinus of the penis since our microstimulation studies indicated pressure changes in the corpus cavernous correlates well with penile erection. In our most recent studies we tried to inject the virus directly into the cavernous sinus. Under halothane anesthesia a small incision was made to the skin overlying the dorsal surface of the penis. From our experience in cannulating the cavernous sinus in our stimulation experiments it was possible to inject a large fraction of the cavernous sinus with virus without making an incision into the body of the penis. Since a septum separates the corpus cavernous on one side from the other, injections were made bilaterally.

Labeled neurons were found on all segments of the sacral spinal cord with the majority in rostral S_2 and caudal S_1 (also see Neuroscience abstract attached to this progress report).

Neurons were located in the area of sacral parasympathetic nucleus, dorsal commissure, and deep in the ventral horn in an area which appears to be part of Onuf's nucleus. Onuf's nucleus contains not only neurons which control external urethral sphincter, but also motoneurons concerned with sexual functions - the bulbocavernosus and ischiocavernosus striated muscle. Microstimulation sites which give the largest increases in cavernous sinus pressure seem to overlap with neurons in the lower to middle part of the ventral horn and with the processes which are projecting toward Onuf's nucleus (See Fig. 1). In other experiments, sites which produce large pressure changes were located somewhat more dorsal in the ventral horn possibly from fibers projecting from interneurons in the dorsal commissure or the sacral parasympathetic nucleus. Examining the results from our microstimulation experiments with information about the location of important neuronal populations from our tracing studies, provides a powerful tool for planning future microstimulation studies.

These types of studies will continue in the next quarter with additional studies on external urethral sphincter neurons as well as hindlimb flexor and extensor motoneurons.

IV. Construction and Assembly of the Components Needed for Motor Studies of the Hindlimb Flexors and Extensors

During this quarter we have completed construction of the spinal frame and leg holder to measure rotational torque at the knee joint to spinal cord stimulus.

Following our last two penile experiments we have used these animals to practice the additional surgery necessary for hindlimb experiments. These animals have also been placed in the apparatus with only a few minor problems which were solved by modification of the apparatus. The computer hardware, software and interfacing seem to be functioning as expected. Plans are to perform an experiment with the primary goal of recording changes in hindlimb

torque to ventral root stimulation and spinal cord microstimulation, in the next few weeks. These studies will continue throughout the next quarter.

Figure 1. Chart recorder output showing the changes in penile pressure (top trace) and bladder pressure (bottom trace) to microstimulation of the S_1 spinal cord at various depths from the surface of the cord for three electrode tracts (A, B and C). The figurine at the bottom right is a transverse section of the S_1 cord showing the position of each tract (A, B and C) and corresponds to the physiological responses shown in each panel labeled A, B and C. The spinal cord was stimulated at 200 μ increments along each tract. Many sites above and below the actual sites produced no effects and these negative sites are not shown in the figure. The shaded mark at the bottom of each panel indicates the occurance of stimulation while the number below each mark is the depth in mm from the surface of the spinal cord for each tract. The stimulus parameters are negative first, balanced pulses at 25 Hz, 0.2 msec duration, 150 μA, applied for 1 min. In C the stimulation at 1.8 mm is repeated. Notice that the sites which give the best response are in the middle and lower half of the ventral horn. These site often extend a few mm in the rostral and caudal direction. In some experiments sites slightly more dorsal also gave good responses.

Figure 2. Chart recorder output showing the change in penile pressure (top trace) and bladder pressure (bottom trace) to various intensities (50-150 μA) of focal stimulation of the S_1 spinal cord with a fine-tipped activated iridium microelectrode. Stimulation parameters are negative first, balanced pulses, 25 Hz, 0.2 msec pulses applied for 1 min. The occurance of stimulation is marked by the shaded marks at the bottom of the figure. Penile pressure was recorded from a catheter placed in the cavernous sinus.

Figure 3. Chart recorder output showing the changes in penile pressure (top trace) and bladder pressure (bottom trace) evoked by various frequencies (15-100 Hz) of stimulation in the S_1 spinal cord with fine-tipped, activated iridium microelectrodes. Stimulus parameters were negative first, balances pulses, 0.2 msec duration, 150μA, for 1 min. Stimulus occurrence is represented by the shaded marks at the bottom of the figure. Penile pressure is recorded from a catheter placed in the cavernous sinus. Notice that at 15 & 20 Hz there is no response to cord stimulation. The peak response was seen between 25 & 35 Hz and slowly decreased in amplitude and duration as the stimulus frequency was increased. Similar results were obtained with ventral root stimulation. Notice also that stimulus evoke bladder responses were small at this level of the spinal cord. It is interesting to note that bladder activity was evoked best at frequencies below 20 Hz.

Figure 1.

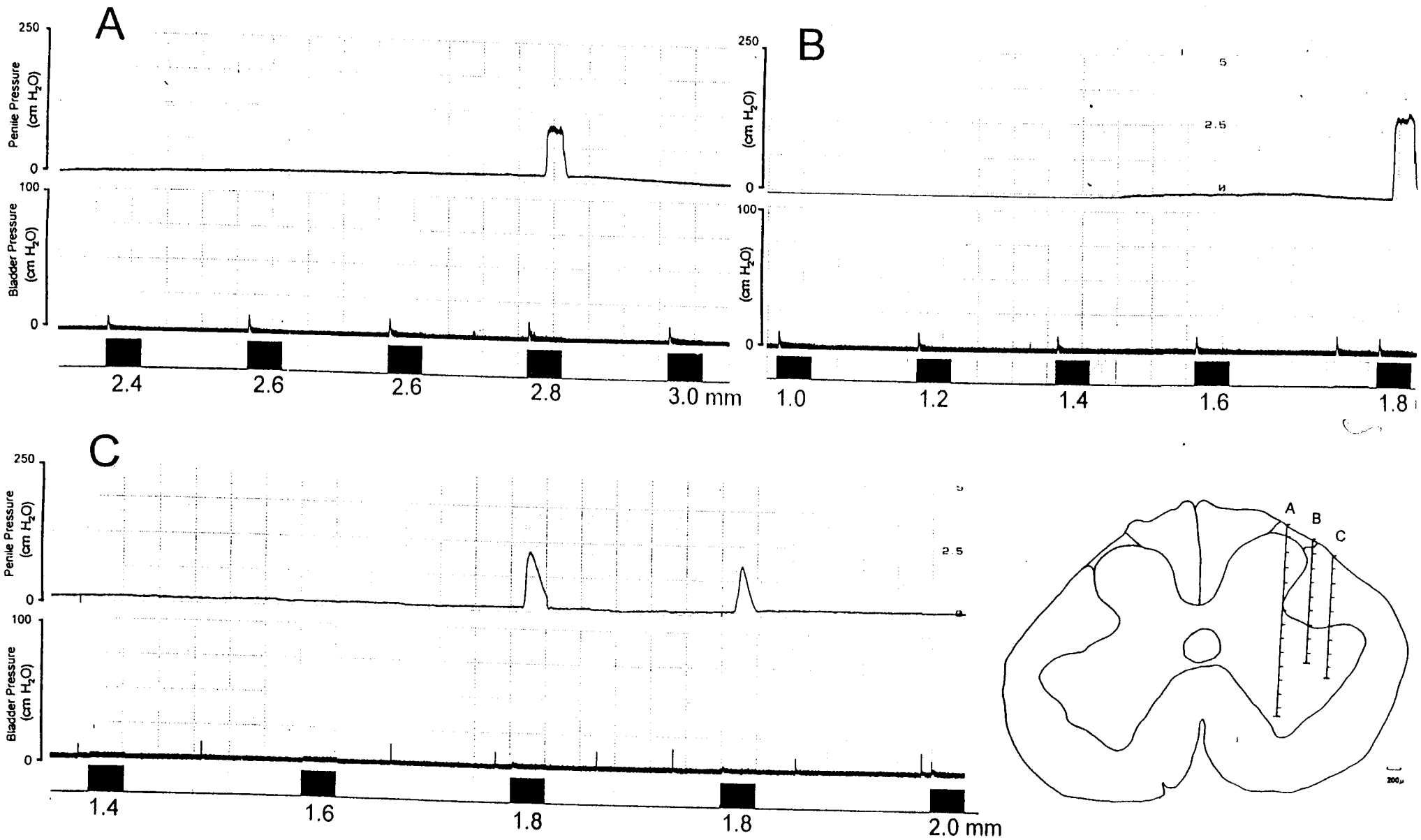

A
Penile Pressure (cm H2O)
250
0
Bladder Pressure (cm H2O)
100
0
2.4
2.6
2.6
2.8
3.0 mm
B
250
(cm H2O)
0
5
2.5
0
100
(cm H2O)
0
1.0
1.2
1.4
1.6
1.8
C
Penile Pressure (cm H2O)
250
0
2.5
0
Bladder Pressure (cm H2O)
100
0
1.4
1.6
1.8
1.8
2.0 mm
A
B
C

Figure 2.

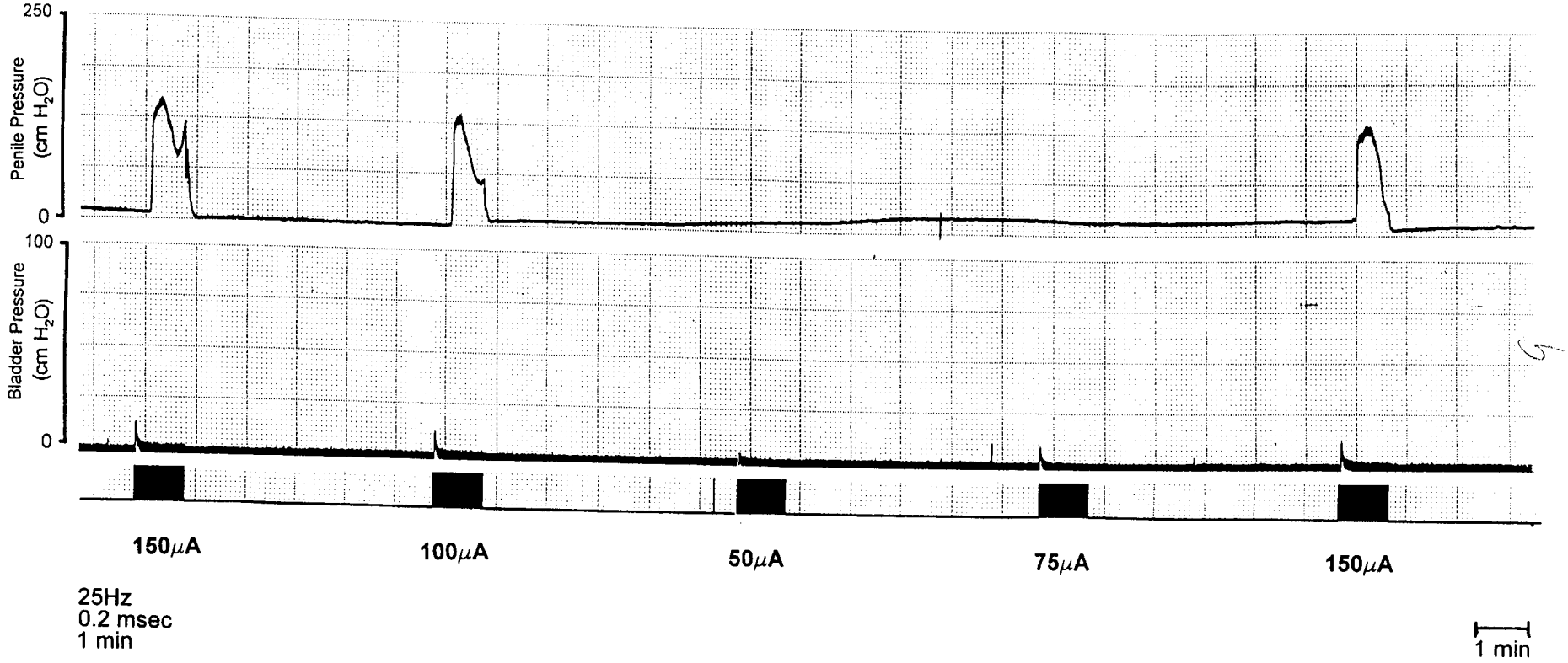
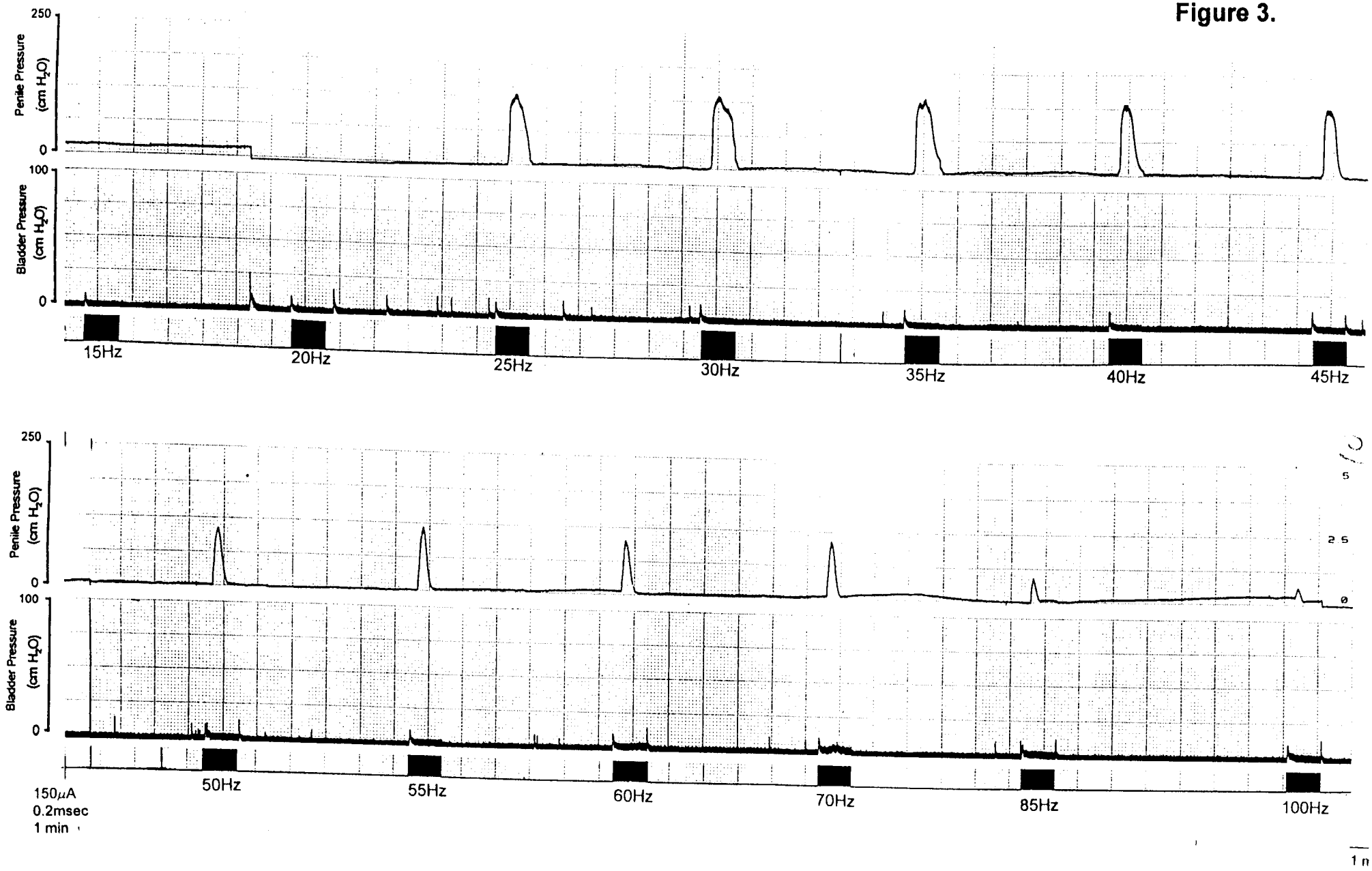

250
Penile Pressure
(cm H_2O)
0
100
Bladder Pressure
(cm H_2O)
0
150μA
100μA
50μA
75μA
150μA
25Hz
0.2 msec
1 min
1 min

Figure 3.


250
Penile Pressure
(cm H_2O)
0
100
Bladder Pressure
(cm H_2O)
0
15Hz
20Hz
25Hz
30Hz
35Hz
40Hz
45Hz
250
Penile Pressure
(cm H_2O)
0
100
Bladder Pressure
(cm H_2O)
0
150μA
0.2msec
1 min
50Hz
55Hz
60Hz
70Hz
85Hz
100Hz
5
2 5
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MODULATION OF CAVERNOUS SINUS PRESSURE (CSP) BY MICROSTIMULATION OF THE SACRAL SPINAL CORD. C. Tai*, A.M. Booth, W.C. de Groat and J.R. Roppolo. Dept. of Pharmacology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261

The purpose of the present study was to determine the stimulation parameters and the location of sites within the sacral spinal cord where focal electrical stimulation produced penile erection in the cat. Since electrically evoked penile protrusion correlated well with increased CSP, pressure recordings from the cavernous sinus (CS) were used as a quantitative measure of penile tumescence or erection. Adult male cats anesthetized with pentobarbital (20-25 mg/kg IV) were used in this study. CSP was recorded via a 22 gauge intracath placed in the CS through a small incision at the tip of the penis. Each sacral ventral root (S_1-S_3) was stimulated with a hook electrode to determine the spinal segment which produced the largest amplitude CSP response. In most cases (5 of 6 animals) the S_1 root produced the largest CS response (100-160 cm H_2O peak pressure), while in one animal the S_2 root was more effective. The S_2 root, however, usually produced the largest bladder response. The segment which produced the largest CSP change was probed with fine tipped (200-400μ^2 surface) activated iridium microelectrodes advanced from the dorsal surface of the spinal cord in 200μ increments. Stimulation sites which produced the largest CS responses were in the middle of the ventral horn, 1.6-2.0 mm from the surface of the S_1 sacral segment and midway between the midline and the lateral edge of the grey matter. The area was 200-400μ wide (medial to lateral) and extended 1-2 mm in the rostrocaudal direction. The response evoked with spinal stimulation occurred at a latency of 8-40 seconds and had an amplitude ranging from 20-120 cm H_2O. The duration and frequency of stimulation was important to produce a maximal response. The stimulus was presented for 1-1.5 minutes. The optimal frequency was between 25-35 Hz using 0.2 msec pulses at an intensity of 50-150μA. These studies suggest that focal microstimulation of the sacral spinal cord may be a useful technique for producing penile erection. (Supported by NO1-NS-5-2332)

Key Words: (see instructions p. 4)

1. Penis
2. parasympathetic
3. Mapping
4. Sex

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